3-1-1992

Endogenous Viral Genes in Thirteen Highly Inbred Chicken Lines and in Lines Selected for Immune Response Traits

Susan J. Lamont
Iowa State University, sjlamont@iastate.edu

Y. Chen
Iowa State University

H. J. M. Aarts
Center for Poultry Research and Information Services

M. C. van der Hulst-van Arkel
Center for Poultry Research and Information Services

G. Beuving
Center for Poultry Research and Information Services

See next page for additional authors

Endogenous Viral Genes in Thirteen Highly Inbred Chicken Lines and in Lines Selected for Immune Response Traits

Abstract

Thirteen highly inbred lines of chickens of Leghorn, Spanish, and Egyptian Fayoumi origin, four partly inbred Leghorn lines selected for MHC alleles and immune response to GAT (*Ir-GAT*), and two replicated, noninbred Leghorn lines divergently selected for multiple immune response traits were subjected to molecular genotyping for endogenous viral (*ev*) gene sequences. In all highly inbred lines of Leghorn origin, *ev* 1 alone or both *ev* 1 and *ev* 2 were observed. The Spanish and Fayoumi lines had three and five *ev* genes, respectively, most of which were not readily identifiable with standard Leghorn *ev* gene loci. The Leghorn lines selected for MHC and *Ir-GAT* had *ev* 1 fixed in the population. Differences in *ev* 3 and *ev* 5 gene frequency were associated with *Ir-GAT* in the *B*1 haplotype, but not in the *B*19 haplotype. In the noninbred lines, which were divergently selected for multiple traits of immune responsiveness, *ev* 6 and *ev* 9 differed in frequency between lines, and both were in lower frequency in the lines selected for high immunoresponsiveness. These two *ev* genes are the only ones known in White Leghorns that have the gs– chf+ phenotype [expressing chicken helper factor (chf) but not expressing group-specific antigen (gs)].

Keywords

endogenous viral genes, chickens, immune response, major histocompatibility complex, inbreeding

Disciplines

Agriculture | Genetics | Immunity | Poultry or Avian Science

Comments

This article is published as Lamont, S. J., Y. Chen, H. J. M. Aarts, M. C. Van Der Hulst-Van Arkel, G. Beuving, and F. R. Leenstra. "Endogenous viral genes in thirteen highly inbred chicken lines and in lines selected for immune response traits." *Poultry Science* 71, no. 3 (1992): 530-538. DOI: 10.3382/ps.0710530. Posted with permission.

Authors

Susan J. Lamont, Y. Chen, H. J. M. Aarts, M. C. van der Hulst-van Arkel, G. Beuving, and F. R. Leenstra

Endogenous Viral Genes in Thirteen Highly Inbred Chicken Lines and in Lines Selected for Immune Response Traits[1]

S. J. LAMONT[2,3] and Y. CHEN

Department of Animal Science, Iowa State University, Ames, Iowa 50011

H.J.M. AARTS, M. C. VAN DER HULST-VAN ARKEL, G. BEUVING, and F. R. LEENSTRA

Center for Poultry Research and Information Services, "Het Spelderholt", Spelderholt 9, 7361 DA Beekbergen, The Netherlands

(Received for publication December 19, 1990)

ABSTRACT Thirteen highly inbred lines of chickens of Leghorn, Spanish, and Egyptian Fayoumi origin, four partly inbred Leghorn lines selected for MHC alleles and immune response to GAT (*Ir-GAT*), and two replicated, noninbred Leghorn lines divergently selected for multiple immune response traits were subjected to molecular genotyping for endogenous viral (*ev*) gene sequences. In all highly inbred lines of Leghorn origin, *ev*1 alone or both *ev*1 and *ev*2 were observed. The Spanish and Fayoumi lines had three and five *ev* genes, respectively, most of which were not readily identifiable with standard Leghorn *ev* gene loci. The Leghorn lines selected for MHC and *Ir-GAT* had *ev*1 fixed in the population. Differences in *ev*3 and *ev*5 gene frequency were associated with *Ir-GAT* in the B^1 haplotype, but not in the B^{19} haplotype. In the noninbred lines, which were divergently selected for multiple traits of immune responsiveness, *ev*6 and *ev*9 differed in frequency between lines, and both were in lower frequency in the lines selected for high immunoresponsiveness. These two *ev* genes are the only ones known in White Leghorns that have the gs^-chf^+ phenotype [expressing chicken helper factor (chf) but not expressing group-specific antigen (gs)].
(*Key words*: endogenous viral genes, chickens, immune response, major histocompatibility complex, inbreeding)

1992 Poultry Science 71:530–538

INTRODUCTION

Endogenous viral (*ev*) genes, which bear sequence homology to avian leukosis virus (ALV), have been identified in almost all lines of chickens. The *ev* genes behave in a Mendelian fashion and are distributed throughout the genome. Depending upon the locus, the transcription may be: 1) complete, leading to production of infectious Subgroup E virus; 2) partial, producing group-specific antigen (gs) or envelop protein (chick helper factor, chf); or 3) silent (Rovigatti and Astrin, 1983). Expression of viral proteins can reduce immune response to exogenous ALV and interfere with testing for exogenous infection in a flock (Crittenden *et al.*, 1984; Gavora, 1986). Detrimental effects may result in altered frequencies of *ev* genes in lines selected for production or

[1]Journal Paper Number J-14310 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA; Project Numbers 2576, 2351.

[2]Research conducted while S. J. Lamont was on sabbatical leave at "Het Spelderholt".

[3]To whom correspondence should be addressed at: Department of Animal Science, 201 Kildee Hall, Iowa State University, Ames, IA 50011.

disease-resistance traits (Kuhnlein *et al.*, 1989a,b; Gavora *et al.*, 1989).

The *ev* genes can be described by the sizes of proviral chicken DNA junction fragments generated after restriction enzyme digestion of genomic DNA and hybridization with probes containing *ev* sequences (Smith, 1986). The restriction fragment length polymorphisms (RFLP) can be used as genetic markers. Although the lack of fixation in most populations suggests that some *ev* loci seem to have advantageous or deleterious effects (Iraqi *et al.*, 1991), the persistence and segregation ratios suggest that most *ev* genes are selectively neutral under normal conditions (Bumstead *et al.*, 1987).

The *ev* genes have been most thoroughly studied in White Leghorns, in which more than 20 *ev* loci have been described (Smith, 1986). Analysis of other populations has shown, however, that the variation in *ev* genes within the species is much greater than that within the White Leghorn type alone (Smith, 1986; Boichard *et al.*, 1990; Aarts *et al.*, 1991; Boulliou *et al.*, 1991; Iraqi *et al.*, 1991; Ronfort *et al.*, 1991).

In the present study, *ev* genes were analyzed in 13 highly inbred lines of chickens, which included three lines unrelated to Leghorns, and in Leghorn lines selected for immune response traits. Specific aims were 1) to identify new *ev* genes; 2) to determine the effects of inbreeding and of selection for general traits of immune response on *ev* loci; and 3) to compare these data with similar, independent experiments to identify *ev* loci that respond to selection for fitness-related traits.

MATERIALS AND METHODS

Chickens

Three sets of chickens were used in these studies. Set 1 consisted of 13 highly inbred chicken lines (Table 1). Inbreeding, calculated from pedigree records, ranged from 98 to 99%. Ten of the highly inbred lines were developed from commercial Leghorns originating from poultry breeders in the United States. One line originated from eggs imported from Spain and two lines originated from Egyptian Fayoumis, which were imported because of anecdotal resistance to the leukosis complex (A. W. Nordskog, 1981, Iowa State University, Ames, IA 50011, personal communication). The Fayoumi lines are also the first genetic stocks in which Rous sarcoma virus resistance on the chorioallantoic membrane was observed (Prince, 1958). Ten samples each of Lines G-B1 and G-B2, representing all effective breeders, were analyzed. Of the other highly inbred lines, three samples each, representing a sire, dam, and one offspring, were tested. Set 2 originated from a cross of commercial Leghorn lines and consisted of four partly inbred sublines, selected initially for MHC haplotype (B^1 or B^{19}) and then, within MHC type, for *Ir-GAT*. A total of 32 samples were tested. Eight individual samples of each of the four sublines were analyzed, representing a sire, dam, and two progeny from each of two different families. Set 3 consisted of two replicated, noninbred lines divergently selected from the Ottawa Strain 7 for multiple immune response traits. Replicate lines were simultaneously selected for two generations for immune response traits of antibody production to *Pasteurella multocida* and *Mycoplasma gallisepticum*, cell-mediated immunity measured by phytohemagglutinin wing-web injection, and clearance of colloidal carbon from the circulatory system. Twenty samples were tested, representing a tail analysis of the most extreme phenotypes from each of the replicated lines; that is, the highest of the high lines, and the lowest of the low lines. Breeder hens in all three sets tested free of ALV shedding into the egg albumin, as monitored by commercial ELISA for p27 antigen.[4]

Genomic DNA Isolation

About 40 μL of whole blood was mixed with 100 μL phosphate-buffered saline containing 150 U/mL heparin. Blood cells were lysed by incubating at 56 C overnight in .5 mL saline-Tris-EDTA (10 m*M* Tris-Cl, pH 8.0, 100 m*M* NaCl, 1 m*M* EDTA, pH 8.0) containing .5% SDS, 60 μg proteinase K, and 20 μg ribonuclease A. The aqueous phase of the lysate was extracted with the phenol-

[4]Idexx, Portland, ME 04104.

TABLE 1. Description of Iowa State University (ISU) chicken lines

Set	Line	Breed	Sublines	B blood group[1]	n	Year of origin	Inbreeding coefficient	Comments
1[2]	8	Leghorn	15.1	42	3	Pre-1940	98	Carries sex-linked barring, originated from crosses of 1930s ISU inbred lines
	19	Leghorn	13 15.1	46 45	3 3	Pre-1940	98	Originated from crosses of 1930s ISU inbred lines
	HN	Leghorn	12 15	12 15	3 3	1954	99	Obtained as pure Kimber line from Heisdorf and Nelson, Inc., (Redmond, WA 98052)
	GH	Leghorn	1 13 15.1	49 48 47	3 3 3	1954	98	Descendants of commercial outbred female from Ghostley Hatchery (Minnesota) crossed to HN male
	G	Leghorn	6 (G-B2) 13 (G-B1)	6 50	10 10	1965	99	Subline of GH, recently rederived as MHC-congenic sublines
	Sp	Spanish	21.1	41	3	1954	98	Originated in Spain
	M	Fayoumi	5.1 15.1	44 43	3 3	1954	98	Originated in Egypt, imported because of anecdotal high resistance to leukosis
2[3]	S1	Leghorn	1H 1L 19H 19L		8 8 8 8	1964	50	Originated from crosses of two Hy-Line® International (Dallas Center, IA 50063) inbred lines, maintained as sublines homozygous for B blood group (1 or 19) and *Ir-GAT* (H or L)
3[4]	IR	Leghorn	1H 2H 1L 2L		5 5 5 5	1988	±3	Originated from Ottawa control Strain 7, replicates (1 and 2) selected for multitrait immune response (high or low, H or L)

[1]B blood group nomenclature of inbred lines (Set 1) proposed by W. E. Briles, 1991 (Northern Illinois University, DeKalb, IL 60115).

[2]Lee, 1980; Knudtson and Lamont, 1989; Warner *et al.*, 1989.

[3]Pevzner *et al.*, 1978; Nordskog and Cheng, 1988.

[4]Cheng *et al.*, 1991.

chloroform-isoamylalcohol procedure (Maniatis *et al.*, 1982), precipitated with ethanol, briefly dried, and dissolved in 100 μL Tris-EDTA buffer.

DNA Analysis

Seven micrograms of chicken genomic DNA were digested overnight to completion (10 units enzyme per 1 μg DNA) with *Sst*1 or *Bam*HI[5] restriction enzyme. The DNA fragments were separated on a .7% TAE-agarose gel [Tris-acetate-EDTA (TAE) buffer: 40 m*M* Tris-acetate, 2 m*M* EDTA] and blotted to a nylon filter[6] by capillary action. Hybridization and washing conditions were per filter manufacturer's recommendations. Briefly, after overnight hybridization at 65 C with the appropriate probe, the filters were washed twice with 2 × saline-sodium citrate (SSC) and .1% SDS at 65 C for 30 min and twice with .1 × SSC and .1% SDS at 65 C for 20 min. The *Hin*dIII-cut λ-DNA was used as a marker to estimate the size of the hybridizing fragments.

Probes

The entire linearized *Eco*RI-digested RCAS plasmid was labeled with [^{32}P] adenosine triphosphate (ATP)[7] and 25 ng used per hybridization to detect *ev* gene-specific sequences. The RCAS plasmid is a Rous sarcoma-derived proviral vector (Hughes *et al.*, 1987). The entire linearized pGdIII probe (25 ng per hybridization), containing the *ev*1 flanking region, was used to identify *ev*1 loci (Hishinuma *et al.*, 1981). The RCAS and pGdIII probes were generously provided by L. B. Crittenden and E. J. Smith [Avian Disease and Oncology Laboratory (formerly Regional Poultry Research Laboratory), East Lansing, MI 48823, and S. H. Hughes, National Cancer Institute, Frederick Cancer Research Institute, Frederick, MD 21701].

Statistical Analysis

The *ev* gene frequency differences were tested for significance by chi-square analysis. Comparisons among sublines were made within each *ev* gene.

RESULTS

Set 1: Highly Inbred Lines

The DNA samples of all breeding animals from the G-B1 and G-B2 lines were probed with the RCAS probe to identify all *ev* genes present. There was no variation in junction fragment pattern within or between these two inbred lines, which had junction fragments for *ev*1 and *ev*2 (not shown). All highly inbred lines of commercial Leghorn origin had both *ev*1 and *ev*2, except for two lines (Lines 8 and HN-B^{15}) that had only *ev*1. Representative lines are shown in Figure 1. The internal *Bam*HI digestion fragments are clearly shown in all lines. The *ev*1-specific flanking region probe (not shown) revealed only the occupied site for all inbred Leghorn lines, except for two lines (GH-B^{13} and GH-$B^{15,1}$) that showed both occupied and unoccupied sites for *ev*1. The Spanish line had junction fragments for three *ev* loci. The two Fayoumi lines presented unique sets of junction fragments (Table 2) not readily identifiable with standard *ev* gene loci as defined in Leghorns. The two Fayoumi lines had a total of five *ev* junction fragments, with four fragments shared between the lines. Segregation patterns defined two of the unique loci. One was identified by an *Sst*I fragment of 6.0 kb and a *Bam*HI fragment of 13 kb, and the other with *Sst*I and *Bam*HI fragments of 23 and 4.9 kb, respectively. Although both sublines of Fayoumis had junction fragments near the size usually associated with *ev*1 (*Sst*I 9.5, *Bam*HI 5.2 kb), the *ev*1-specific probe hybridized only with an *Sst*I fragment of 13 kb, which is different than that usually associated with an *ev*1 occupied site (Figure 2).

Set 2: Lines Selected for Major Histocompatibility Complex Type and Immune Response to Glutamic Acid-Alanine-Tyrosine

The presence of *ev*1 was fixed in the Leghorn lines selected for MHC and *Ir-GAT*. Both *ev*3 and *ev*5 were segregating in the lines (shown in DNA pools from B^1

[5]Gibco BRL; Middlesex, England.
[6]Hybond-N, Amersham, Houten, England.
[7]Multi-prime, Promega, Madison, WI 53711.

TABLE 2. Size (kilobases) of endogenous virus (*ev*)-related bands in Spanish and Fayoumi lines

Spanish		Fayoumi			
		Subline 5.1		Subline 15.2	
*Sst*I	*Bam*HI	*Sst*I	*Bam*HI	*Sst*I	*Bam*HI
21	5.2	. . .	19	23	19
9.5	4.4	13	13	13	. . .
4.2	1.5	9.4	9.4	9.4	9.4
		8.5	8.5	8.5	8.5
		6.0	8.0	. . .	8.0
		5.4	5.2	5.4	5.2
		. . .	. . .	. . .	4.9

haplotype birds in Figure 1). In the B^1 haplotype, *ev*3 was present in most *Ir-GAT*^{High} birds, and absent from most *Ir-GAT*^{Low} birds. The reverse was the case for *ev*5. There was no observable association of *ev*3 and *ev*5 with *Ir-GAT* within the B^{19} haplotype.

Set 3: Lines Selected for Multiple Immune Response Traits

The *ev* genes in the lines selected for high or low composite immune response can be compared with the control Strain 7 *ev* gene frequencies (Kuhnlein *et al.*, 1989b). The animals evaluated in the selected lines represented a tail distribution: that is, the lowest phenotypes from the low line and the highest phenotypes from the high line. Table 3 shows that *ev*1 is fixed in this population. Two *ev* genes, *ev*6 and *ev*9, differed significantly in frequency between the high- and low-selected lines, with the control Strain 7 frequency of *ev*6 and *ev*9 being intermediate to that of the selected lines.

DISCUSSION

Set 1: Highly Inbred Lines

Analysis of the highly inbred lines of Set 1 should be divided into two groups: lines of Leghorn origin and those of Spanish and Fayoumi origin, inasmuch as these two groups present quite different patterns. All individuals of the lines of Leghorn origin contained only one (*ev*1) or two (*ev*1 and *ev*2) *ev* genes, reflecting their extremely high level of inbreeding (98 to 99%) or common ancestry from United States commercial layer stock of the 1930s to 1950s (Table 1). This confirms the almost universal distribution of the *ev*1 locus in United States commercial layers (Tereba and Astrin, 1980). The lack of within- and between-line variation in the G-B1 and G-B2 lines supports the homogeneity of the background genome of these MHC-congenic lines. The cause of the persistence of both occupied and unoccupied sites for *ev*1 in the highly inbred GH-B^{13} and GH-$B^{15.1}$ sublines is unknown, and may be due to either heterozygosity for *ev*1, or heterogeneity in the *ev*1 locus. It may relate to the outcross of these lines about 15 yr ago, to incorporate the B^1 haplotype into the GH line for study of its effects. After the outcross, the separate sublines based upon MHC type were formed. Fingerprint studies with microsatellite probes on the inbred lines also suggest persistence of heterozygosity in the GH sublines (Plotsky, Kaiser, and Lamont, unpublished data).

The non-Leghorn inbred lines had a greater number of *ev* junction fragments than the Leghorn lines. The Spanish line appeared to have *ev*1, but the other two loci were not readily identifiable with standard Leghorn-defined *ev* genes (Smith, 1986). The *ev*1 locus of the Fayoumi lines presents

TABLE 3. Analysis of immune response-selected line endogenous virus (*ev*)-related bands: frequency of bands by sublines

	Subline				
	Control[1]	Selected			
ev locus	7 (n = 33)	High (n = 10)		Low (n = 10)	
1	1.00	1.00		1.00	
3	.61	.50		.40	
5	.58	.80		.70	
6	.48	.30	*	.70	
9	.27	.20	*	.50	
4	.09	.00		.00	
7	.06	.00		.00	
8	<.03	.10		.00	

[1]From Kuhnlein *et al.*, 1989b.

*Frequencies of *ev* locus differ between selected lines (P<.05).

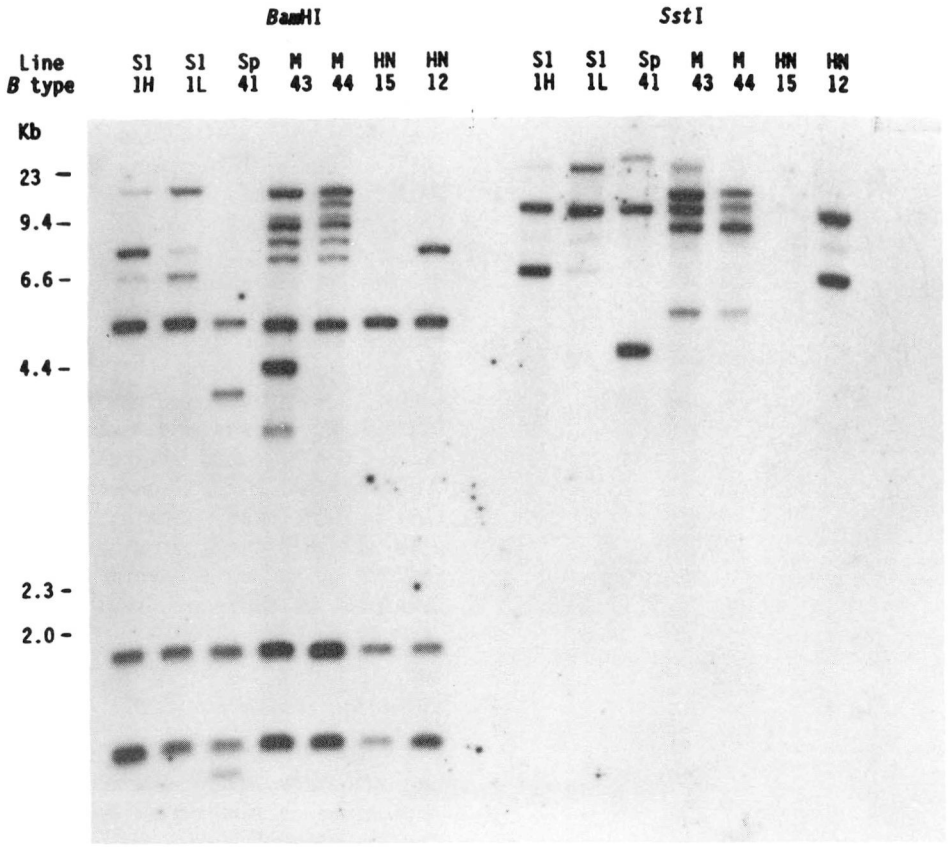

FIGURE 1. Autoradiograph of DNA hybridized with the RCAS probe, a Rous-sarcoma-derived proviral vector. The DNA sources are indicated by line and B blood group type. All lanes represent DNA from one individual, except S1 lanes, which contain pools from eight individuals. Samples from the same birds are used for the *Bam*HI and *Sst*I digestions. Molecular weights are indicated in kilobases. The sample in *Sst*I Lane HN-15 was mistakenly added at too low a concentration to visualize results here.

a unique situation. Junction fragments of nearly typical *ev*1 size (*Sac*I, 9.5 and *Bam*HI, 5.2 kb) are seen. On hybridization with the *ev*1 flanking probe, however, only the 13-kb fragment length typical of the unoccupied *ev*1 site (Smith and Crittenden, 1986) is seen (Figure 2b, Lane 3). Therefore, either the *ev*1 gene is present and the fragments are of a greater size in the Fayoumis than in the Leghorns, or the Fayoumis do not have *ev*1 and the fragments seen of the typical *ev*1 size actually represent other *ev* loci. It is hoped that study of the *ev* genes in this line may shed some light on the anecdotal properties of leukosis resistance, which motivated the original importation of these birds to North America. In a study conducted in 1980 and 1981, the $B^{15.1}$ haplotype of the Fayoumi displayed resistance to challenge with Marek's disease virus that was superior to that of most of the Leghorn lines studied (A. W. Nordskog, Iowa State University, Ames, IA 50011, personal communication, 1981). Boichard *et al.* (1990) also analyzed *ev* genes in Fayoumi chickens. They found a higher number of loci (14) and marked heterogeneity. Several of the junction fragments identified in the present study seem to correspond with those present in the highest frequencies in the Boichard *et al.* (1990) study.

Analysis of *ev* genes as representative marker loci for evaluating genetic diversity can be compared with a study on these same lines utilizing another DNA probe for RFLP, an MHC Class IIβ probe (Warner *et*

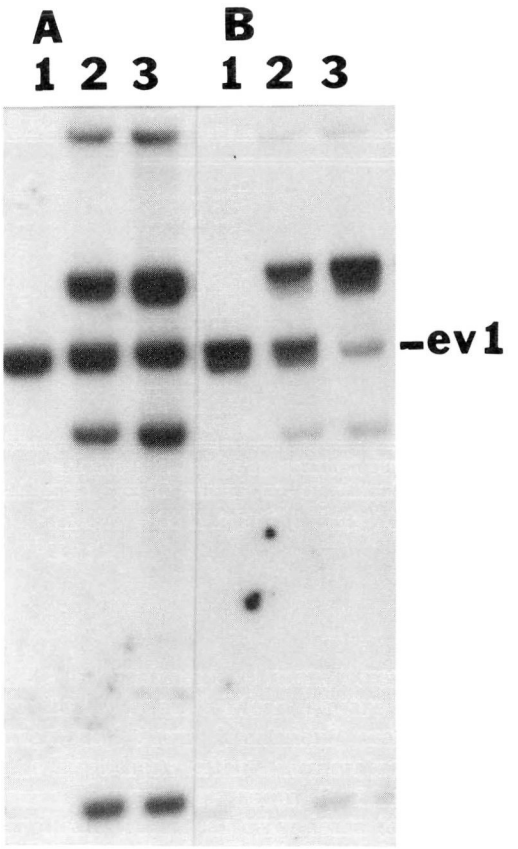

FIGURE 2. Autoradiograph of *Sst*I-digested DNA hybridized with a) the Rous sarcoma-derived proviral vector, RCAS probe; or b) an endogenous viral gene *ev*1-flanking probe. Samples are from 1) Line HN-B^{15}; 3) Line M-B^{43}; or 2) a cross between the two lines. The position of the *ev*1 occupied site (9.4 kb) is indicated.

al., 1989). In agreement with the present study, the MHC probe showed homogeneity of RFLP pattern within each inbred line, and the Spanish and Fayoumi lines had patterns distinct from the lines of Leghorn origin.

Set 2: Lines Selected for Major Histocompatibility Complex Type and Immune Response to Glutamic Acid-Alanine-Tyrosine

The birds of Set 2 all exhibited *ev*1, but the four genotypes varied in frequency of *ev*3 and *ev*5. The small number of genes may be reflective of the development of this line, which included selection first for MHC type, then for *Ir-GAT* type within MHC type, and partial (50%) inbreeding (Pevzner *et al.*, 1978; Nordskog and Cheng, 1988). There is a significant difference in the frequency of *ev*3 and *ev*5 associated with *Ir-GAT* in the B^1 birds; most B^1-*IrGAT*^{High} birds have *ev*3 and most B^1-*IrGAT*^{Low} birds have *ev*5. This association may be due to the presence of both *Ir-GAT* and the *ev*3 locus on the MHC-bearing microchromosome (Pevzner *et al.*, 1978; Plachý *et al.*, 1985) or to founder effects in this partially inbred line (Nordskog and Cheng, 1988). Because the B^1-*IrGAT*^{High} linkage arose from a single individual from a cross between the parental B^1-*IrGAT*^{Low} and B^{19}-*Ir-GAT*^{High} types (Pevzner *et al.*, 1978), the association with *Ir-GAT* is more easily seen in B^1 birds than with the B^{19} birds from which B^{19}-*Ir-GAT*^{Low} arose from several different individuals in different families.

Set 3: Lines Selected for Multiple Immune Response Traits

As also observed in the highly inbred Leghorn lines, *ev*1 was fixed in the base population, Ottawa Strain 7, of this selection experiment, again confirming the almost universal distribution of this gene in North American Leghorn lines (Table 3). Kuhnlein *et al.* (1989b) identified eight different *ev* loci in the Control Strain 7. Two of the *ev* loci (*ev*4 and *ev*7) present in very low frequency in the control line did not appear in the selected lines of the present study. This may be due to their loss from the selected lines or because they were not present in the sample (n = 10) taken from each line. Although the number of birds analyzed in each selected line was not large, they were selected to represent a "tail distribution" of extreme phenotypes to optimize the chance of detecting differences generated by the selection procedure. The selection for multiple immune response traits was associated with significant differences between the selected lines in frequency of two *ev* loci. The two *ev* genes, *ev*6 and *ev*9, are the only two known in White Leghorns with the gs$^-$chf$^+$ phenotype (Smith, 1986). Both *ev*6 and *ev*9 were present in a lower frequency in the high-response line. This provides independent confirmation of the observation of Kuhnlein *et al.*

(1989b) of a decreased frequency of these loci in lines selected for resistance to Marek's disease. Thus, studies in separate laboratories with different selection criteria related to disease resistance and immune response have demonstrated a similar decrease in frequency in *ev6* and *ev9*, emphasizing the negative association of these two *ev* genes with fitness-related traits. Smith *et al.* (1990a,b; 1991) reported various effects of *ev6* on immune response to ALV, which may illustrate mechanisms for *ev* gene modulation of immunity.

REFERENCES

Aarts, H.J.M., M. C. van der Hulst-van Arkel, G. Beuving, and F. R. Leenstra, 1991. Variations in endogenous viral (*ev*) gene patterns in White Leghorn, medium heavy, White Plymouth Rock and Cornish type chickens. Poultry Sci. 70: 1281–1286.

Boichard, M., L. Durand, C. Printz, H. Leveziel, and G. Dambrine, 1990. Preliminary analysis of endogenous viral genes in Fayoumi and Rhode Island Red chickens. Proceedings 4th World Congr. Genet. Appl. Livest. Prod. (Darling, Penicuik, Midlothian, Scotland) 13:143–146.

Boulliou, A., J. P. LePennec, G. Hubert, R. Donal, and M. Smiley, 1991. Restriction fragment length polymorphism analysis of endogenous avian leukosis viral loci: determination of frequencies in commercial broiler lines. Poultry Sci. 70: 1287–1296.

Bumstead, N., L. I. Messer, and N. G. Greenwood, 1987. Use of *ev* loci as a measure of inbreeding in domestic fowls. Br. Poult. Sci. 28:717–725.

Cheng, S., M. F. Rothschild, and S. J. Lamont, 1991. Estimates of quantitative genetic parameters of immunological traits in the chicken. Poultry Sci. 70:2023–2027.

Crittenden, L. B., E. J. Smith, and A. M. Fadly, 1984. Influence of endogenous viral (*ev*) gene expression and strain of exogenous avian leukosis virus (ALV) on mortality and ALV infection and shedding in chickens. Avian Dis. 28:1037–1056.

Gavora, J. S., 1986. Influences of avian leukosis virus infection on production and morality and the role of genetic selection in the control of lymphoid leukosis. Pages 241–260 *in*: Avian Leukosis. G. F. de Boer, ed. Martinus Nijhoff, Boston, MA.

Gavora, J. S., U. Kuhnlein, and J. L. Spencer, 1989. Absence of endogenous viral genes in an inbred line of Leghorn chickens selected for high egg production and Marek's disease resistance. J. Anim. Breed. Genet. 106:217–224.

Hishinuma, F., P. J. DeBona, S. Astrin, and A. M. Skalka, 1981. Nucleotide sequences of acceptor sites and termini of integrated avian endogenous provirus *ev*1: Integration creates a 6 bp repeat of host DNA. Cell 23:155–164.

Hughes, S. H., J. J. Greenhouse, C. J. Petropoulos, and P. Sutrave, 1987. Adaptor plasmids simplify the insertion of foreign DNA into helper-independent retroviral vectors. J. Virol. 61:3004–3012.

Iraqi, F., M. Soller, and J. S. Beckmann, 1991. Distribution of endogenous viruses in some commercial chicken layer populations. Poultry Sci. 70:665–679.

Knudtson, K. L., and S. J. Lamont, 1989. Associations of genetics and sampling time with levels of interleukin-2 activity. Vet. Immunol. Immunopathol. 22:333–343.

Kuhnlein, U., J. S. Gavora, J. L. Spencer, D. E. Bernon, and M. Sabour, 1989a. Incidence of endogenous viral genes in two strains of White Leghorns chickens selected for egg production and susceptibility or resistance to Marek's disease. Theor. Appl. Genet. 77:26–32.

Kuhnlein, U., M. Sabour, J. S. Gavora, R. W. Fairfull, and D. E. Bernon, 1989b. Influence of selection for egg production and Marek's disease resistance on the incidence of endogenous viral genes in White Leghorns. Poultry Sci. 68:1161–1167.

Lee, W.-H., 1980. Use of Splenomegaly to Characterize the B Complex in Chickens. M. S. thesis, Iowa State University, Ames, IA.

Maniatis, T., E. F. Fritsch, and J. Sambrook, 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Nordskog, A. W., and S. Cheng, 1988. Inbreeding effects on fertility and hatchability associated with the formation of sublines. Poultry Sci. 67: 859–864.

Pevzner, I. Y., C. L. Trowbridge, and A. W. Nordskog, 1978. Recombination between genes coding for immune response and the serologically determined antigens in the chicken B system. Immunogenetics 7:25–33.

Plachý, J., E. Korec, I. Hlozánek, and E. Zdenková, 1985. Genetic linkage of endogenous viral loci with the B (MHC), and C histocompatibility loci in chickens. Folia Biol. (Praha) 31:353–356.

Prince, A. M., 1958. Quantitative studies on Rous sarcoma virus. II. Mechanism of resistance of chick embryos to chorio-allantoic inoculation of Rous sarcoma virus. J. Natl. Cancer Inst. 20: 843–850.

Ronfort, C., M. Afanassieff, Y. Chebloune, G. Dambrine, V. M. Nigon, and G. Verdier, 1991. Identification and structure analysis of endogenous proviral sequences in a Brown Leghorn strain. Poultry Sci. 70:2161–2175.

Rovigatti, U. G., and S. M. Astrin, 1983. Avian endogenous viral genes. Pages 1–21 *in*: Retroviruses 1. P. K. Vogt and H. Koprowski, ed. Current Topics in Microbiology and Immunology Vol. 103. Springer-Verlag, Berlin and Heidelberg, Germany, and New York, NY.

Smith, E. J., 1986. Endogenous avian leukemia viruses. Pages 101–120 *in*: Avian Leukosis. G. F. de Boer, ed. Martinus Nijhoff, Boston, MA.

Smith, E. J., and L. B. Crittenden, 1986. Endogenous viral genes in a slow-feathering line of white Leghorn chickens. Avian Pathol. 15:395–406.

Smith, E. J., A. M. Fadly, and L. B. Crittenden, 1990a. Interactions between endogenous virus loci *ev6*

and *ev*21. 1. Immune response to exogenous avian leukosis virus infection. Poultry Sci. 69: 1244–1250.

Smith, E. J., A. M. Fadly, and L. B. Crittenden, 1990b. Interactions between endogenous virus loci *ev*6 and *ev*21. 2. Congenital transmission of EV21 viral product to female progeny from slow-feathering dams. Poultry Sci. 69:1251–1256.

Smith, E. J., A. M. Fadly, I. Levin, and L. B. Crittenden, 1991. The influence of *ev*6 on the immune response to avian leukosis virus infection in rapid-feathering progeny of slow- and rapid-feathering dams. Poultry Sci. 70:1673–1678.

Tereba, A., and S. M. Astrin, 1980. Chromosomal localization of *ev*-1, a frequently occurring endogenous retrovirus in White Leghorn chickens, by in situ hybridization. J. Virol. 35:888–894.

Warner, C., B. Gerndt, Y. Xu, Y. Bourlet, C. Auffray, S. Lamont, and A. Nordskog, 1989. Restriction fragment length polymorphism analysis of major histocompatibility complex class II genes from inbred chicken lines. Anim. Genet. 20:225–231.